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Identification of Larval Pacific, River, and Western Brook Lampreys and Thermal Requirements of Early Life History Stages of Lampreys

Larval Pacific Lampreys (*Lampetra Tridentata*),
River Lampreys (*L. Ayresi*), and
Western Brook Lampreys

Annual Report 2000



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RIVER LAMPREYS (*L. AYRESI*), AND WESTERN BROOK LAMPREYS (*L.*
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STAGES OF LAMPREYS

ANNUAL REPORT 2000

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EXECUTIVE SUMMARY

Pacific lampreys (*Lampetra tridentata*) in the Columbia River Basin (CRB) are believed to have declined to only a remnant of their population prior to human development, and actions are currently being considered for their recovery (Close et al. 1995). Identifying biological factors that may limit lamprey production in the CRB is critical for their recovery, and while some biological information for this and sympatric species [the western brook lamprey (*L. richardsoni*) and the river lamprey (*L. ayresi*)] is available from studies in Canada (Pletcher 1963, Beamish 1980, Richards 1980, Beamish and Levings 1991), little is known about the biology of lampreys in the CRB.

In order to identify biological factors that may limit lamprey production in the CRB it is important to be able to document the distribution and relative abundance of lampreys in streams and rivers tributary to the Columbia River. This may be accomplished through surveys of ammocoetes (larval lamprey), which can be readily collected from rearing habitat. However, characteristics currently employed to differentiate ammocoetes of different species may not be diagnostic (USGS, unpublished data). Therefore, developing ammocoete identification techniques is critical to determine the distribution and abundance of lampreys.

Along with the ability to distinguish lamprey species in the field, understanding factors influencing survival during early life history stages is important. One such factor is temperature, which may determine ammocoete abundance (Potter and Beamish 1975, Young et al. 1990, Youson et al. 1993). Understanding how temperature affects survival of early life history stages will help identify critical habitat needs that influence lamprey distribution and abundance (Piavis 1961, Holmes and Lin 1994).

The purpose of our research is to provide information necessary for population assessment and determination of critical habitat needs. Currently we have completed the first year of a multi-year study intended to: 1) determine diagnostic characteristics of egg and larval stages of Pacific, western brook, and river lampreys, and 2) examine the effects of temperature on the timing of developmental events and on survival of early life history stages of Pacific, western brook, and river lampreys.

Both adult and ammocoete Pacific and western brook lampreys were collected from the field and transported to the Columbia River Research Laboratory (CRRL). We were unable to locate any river lamprey specimens within the CRB. Half of the ammocoetes were identified and sacrificed to provide genetic samples. We are currently awaiting finalization of genetic analyses, the results of which will give us a preliminary indication of our ability to accurately identify ammocoetes based on current diagnostic characteristic. The remaining ammocoetes were individually marked, held in aquaria, and sampled at six-week intervals. At each sampling event metamorphosis stage was noted and ammocoetes were identified to species, measured for length and mass, and photographed. The purpose of this sampling is to test the validity of current diagnostic techniques by holding ammocoetes until metamorphosis, at which time positive identification can be made. To date we have sampled these ammocoetes seven times, and our species identification of individuals has been relatively consistent over time.

Adult lampreys were spawned and the resulting progeny were reared for two purposes. First, eggs were incubated at four temperatures (10, 14, 18, and 22° C) and eggs and prolarval lampreys were measured (length and mass), photographed, and preserved at intervals over a period of 25 days. The samples collected will provide us

with information about the timing of different developmental stages and the effects of temperature on the timing of developmental stages. The samples collected will also be used to determine morphological differences between Pacific and western brook lampreys and to develop an identification key for lamprey species of the CRB.

Second, we used the progeny of laboratory-spawned lampreys to examine the effects of temperature on survival of early life stage Pacific and western brook lampreys. Both species of lamprey examined showed a marked decrease in survival at 22° C when compared to other temperatures (10, 14, and 18° C). Rate of development increased at warmer temperatures. Thus, it is important to consider the effects of temperature in the context of developmental stage.

This project will be continued in 2001, and we will expand our efforts to locate river lampreys, which were not collected in 2000. We will continue tracking ammocoetes for validating species identification techniques and we will collect more samples for the purpose of developing a key for identifying lamprey species of the CRB. We will also continue to examine the effects of temperature on survival of early life stage lampreys and examine techniques for comparing temperature effects based on developmental stage.

ACKNOWLEDGEMENTS

We thank individuals in the U. S. Fish and Wildlife Service, University of Idaho, Idaho Department of Fish and Game, Confederated Tribes of the Umatilla Indian Reservation, and the U. S. Geological Survey for their assistance with project activities. We also appreciate the assistance of Debbie Docherty, Project Manager, Bonneville Power Administration.

INTRODUCTION

The ecological, economic, and cultural significance of Pacific lampreys (*Lampetra tridentata*) has been underestimated historically (Kan 1975, NPPC 1995, Close et al. 1995) and actions are currently being considered for their recovery in the Columbia River Basin (CRB) (Close et al. 1995). Identifying the biological and ecological factors that may limit lamprey production in the Columbia River Basin is critical for their recovery. Although some biological and ecological information for this and sympatric species [the western brook lamprey (*L. richardsoni*) and the river lamprey (*L. ayresi*)] is available from studies conducted in Canada (Pletcher 1963, Beamish 1980, Richards 1980, Beamish and Levings 1991), little is known about the biology and ecology of lampreys in the CRB (Kan 1975, Hammond 1979).

Documenting the distribution and relative abundance of lampreys in streams and rivers tributary to the Columbia River will help identify factors limiting lamprey populations, identify areas in need of rehabilitation, and help to assess the efficacy of management actions. Surveys of ammocoetes (larval lamprey) may provide an effective means of determining distribution and abundance since ammocoetes are readily collected from rearing areas by electrofishing. However, our inability to differentiate ammocoetes of different species limits the utility of this technique. Identification of Pacific, river, and western brook lamprey ammocoetes is not resolved and characters currently used to differentiate species have proven to not be diagnostic (USGS, unpublished data). Therefore, developing ammocoete identification techniques is critical to determine the distribution and abundance of these lampreys.

Along with the ability to distinguish lamprey species in the field, identification of biological and ecological factors limiting lampreys in the CRB is critical to population assessment and recovery efforts. Understanding factors influencing survival during early life history stages is particularly important since this period is a critical determinant of recruitment to many fish populations (Houde 1987). Ammocoete abundance may be determined by water temperature, or by other physical habitat characteristics, during early development (Potter and Beamish 1975, Young et al. 1990, Youson et al. 1993). For example, the range of optimal temperatures for survival of sea lamprey embryos is narrow (Piavis 1961).

Understanding how temperature affects survival of early life history stages will help identify critical habitat needs that influence lamprey distribution and abundance (Holmes and Lin 1994). Information on the role of temperature in larval lamprey development will provide managers with a means to assess suitability of available spawning and rearing habitats, which may be suboptimal due to alterations in thermal regimes of the Columbia River and its tributaries (Quinn and Adams 1996).

The goal of this project is to address two fundamental aspects of lamprey biology in order to provide tools for population assessment and determination of critical habitat needs of the Columbia River Basin lampreys. In particular, our objectives are to: 1) determine diagnostic characteristics of egg and larval stages of Pacific, western brook, and river lampreys, and 2) examine the effects of temperature on the timing of developmental events and on the survival of early life history stages of Pacific, western brook, and river lampreys.

This work will answer questions about Pacific lampreys posed by regional fishery managers. Specifically, providing tools for population assessment and the quantification of habitat needs will help managers in developing strategies to assure the long-term stability of Pacific lamprey populations and reduce the likelihood that the management of this species will be handled through the regulatory process. Knowledge of the early life history characteristics of these species will aid in future research and management of lampreys in the Columbia River Basin. Accurate identification of ammocoetes will allow managers to conduct larval surveys and thus determine the relative abundance of each species in various habitats. Presence or absence of Pacific lampreys in a given stream will play a key role in lamprey rehabilitation by identifying optimal habitats and locating areas suitable for recovery efforts.

METHODS

Collection, holding, and spawning of lampreys

Pacific lamprey-One hundred adult Pacific lampreys were collected from the John Day Dam during winter maintenance of the north shore adult fishway in December 1999. These lampreys were transported to the Columbia River Research Laboratory (CRRL) and held in 1.52-m-diameter, 1400 L circular tanks provided with a continuous inflow of water (ca. 4-6 L/min) from the Little White Salmon River. Water used for this research was heated to simulate the natural temperature regime of the Columbia River and lampreys were exposed to a simulated natural photoperiod produced by incandescent lights controlled by timers.

Western brook lamprey-Most of the adult western brook lampreys used for spawning in 2000 were animals that had been collected as ammocoetes in 1999 for

another study and held at the CRRL until they metamorphosed. We attempted to collect additional adult western brook lampreys from lower Columbia River tributaries (Chelatchie Creek), but we were unable to find any sexually mature females (all the animals collected from this location were male). Western brook lampreys were held in flow-through aquaria and provided substrate and food. These tanks were exposed to simulated natural temperature and photoperiod regimes as described above for adult Pacific lampreys.

River lamprey-We were unable to locate adult river lampreys. We have contacted staff from state organizations, federal organizations, tribes, universities, and Canadian provinces seeking information on the distribution and availability of river lampreys. To date we have not been successful but will continue this activity through the winter of 2000-2001.

We examined the lampreys periodically through the winter and spring of 1999-2000. Starting in early April, we began to see external morphological changes consistent with observations of secondary sexual characteristics seen in lampreys spawned at the CRRL in 1999 and consistent with published descriptions of secondary sexual characteristics of other lamprey species (Hardisty and Potter 1971). By May 2000, most western brook and some Pacific lampreys displayed the beginnings of the urogenital pore swollen lobe (initially visible as a ridge starting immediately posterior to the urogenital pore). By late May 2000, the female western brook lampreys were all sexually mature. At this time, we were also able to detect the male urogenital papillae. The lampreys became very active, including moving rocks by attaching on with their oral discs, attacking each other, pairing side by side, and attaching to the sides of the tank and

flagellating their entire body with sufficient force to move sand and gravel against the side of the tank.

Mature lampreys were removed from the holding tanks, anesthetized for four minutes in 5 L of 60 mg/L concentration of tricaine methanesulfonate (MS-222) buffered with an equal concentration of sodium bicarbonate. Lampreys were rinsed in fresh water before spawning to remove traces of anesthetic.

Gametes were removed from the lampreys, using different procedures according to sex and species, similar to Piavis (1961). Gametes were stripped from the female lampreys first. Each female lamprey was positioned over a glass bowl filled with about 2 L of fresh water. For western brook lampreys, eggs were forced out the urogenital opening by squeezing the abdomen in a downward motion. This was repeated until blood appeared with the gametes. Male lampreys were stripped of gametes in a similar fashion. For Pacific lampreys, eggs were stripped through an incision along the ventral side from below the gill slits to the vent. Both sperm and eggs were released more readily when the urogenital opening was submerged in the water. Eggs were slightly adhesive when exposed to air, but less so when mixed with water.

Gametes were mixed with a gentle flow of water from a laboratory wash bottle or a large pipette and allowed to rest undisturbed at room temperature for 0.5 h to allow fertilization to occur. After fertilization, the total egg/milt solution was divided into four glass bowls and the water temperature of each bowl was adjusted gradually (approximately 0.5 h), through addition of warm or cool water, until the target temperatures of 10, 14, 18, and 22° C were reached. Once target temperatures were reached, eggs were transferred to flow-through incubators (MacDonald jars) of the

appropriate temperature. Eggs were incubated at 10, 14, 18, and 22° C (\pm 0.5 °C)(one incubator per treatment).

Ammocoete identification

We have taken a two-pronged approach to pursue this objective. We are simultaneously evaluating a published key for identification of older ammocoetes (Richards et al. 1982) and initiating complete morphometric description of these species based on specimens that are the result of captive spawning in the laboratory.

Validation of current diagnostic characteristics

Ammocoetes were collected from five locations in the Columbia River Basin: Red River (Clearwater River subbasin), Entiat River (Snake River subbasin), John Day River, Walla Walla River, and Cedar Creek (Lewis River subbasin). These ammocoetes were collected by cooperators and transported to the CRRL. We received 10-25 ammocoetes from each location. Each ammocoete was measured for length and mass, and identified to species based on existing diagnostic characteristics. Approximately half of the ammocoetes were photographed and sampled to provide tissue for genetic testing (laboratory analysis conducted by Dr. Matt Powell, University of Idaho). This will allow us to confirm the species identification for each individual. These data will be used along with photographs as a means to examine current methodology for ammocoete identification.

The remaining ammocoetes were similarly measured for length and mass, identified to species, and photographed. Photographs were taken to validate current techniques that base species identification on characteristics of the caudal region (Richards et al. 1982). Each ammocoete was given a unique mark using dyed elastomer

and placed in flow-through aquaria provided with a continuous inflow of water (ca. 1-2 L/min) from the Little White Salmon River. Water used for this research was heated to simulate the natural temperature regime of tributaries to the Columbia River and aquaria were exposed to a simulated natural photoperiod produced by incandescent lights controlled by timers. Substrate, food, and air were provided. Ammocoetes were sampled at 6-week intervals. At each sampling event individuals were weighed and measured, identified to species, photographed, and their stage of metamorphosis was determined.

Morphometric description of laboratory spawned specimens

Following fertilization (see above), approximately twenty individuals were removed from each MacDonald jar 4, 8, 16, 20, and 24 d after spawning. Using a large pipette, the eggs/prolarvae (see Piavis 1961) were removed from the MacDonald jar and placed in a petri dish. A dissecting microscope fitted with a polarizing light source, a calibrated ocular micrometer, and a 35 mm camera was used to examine and photograph the eggs and prolarvae. Diameter of yolk and chorion were measured for eggs; notochord length was measured for prolarvae. Photographs were taken of normal and abnormal eggs and prolarvae at each sampling event. Eggs and prolarvae were preserved in either 10% formalin or 95% ethanol following examination.

In the future, this developmental time series of the progeny of each species will be used to prepare conventional species descriptions as in Kendall et al. (1984). This will include completing morphometric measurements and meristic counts of individuals from the laboratory-reared time series of each species. This information will be used to prepare drawings and construct tables of morphometric measurements and ultimately to prepare a dichotomous key to separate these species.

Effects of temperature on early life history stages

Egg incubation and examination procedures were the same for both species spawned in 2000. Following fertilization (see above), eggs were incubated at 10, 14, 18, and 22° C for a minimum of 18 h, allowing the eggs time to reach a developmental stage where it was possible to confirm fertilization had occurred. At this point, 100 fertilized eggs were counted into each of ten eggcups for each temperature and incubated in a water bath of the appropriate temperature for the duration of the experiment. All eggcups were examined daily to determine the number of live, dead (removed), and abnormal individuals present. Observations were made until prolarvae had assimilated approximately 50 percent of their yolk sac.

The effects of temperature on survival are presented as mortality rates based on days at treatment and on accumulated degree-days. We used a linear degree-day model in order to provide a standard metric for comparisons among treatments. Although the base temperature for development is not known for these species, it was possible to use an arbitrary value for the base temperature for our purposes because we were using this metric for comparative purposes (not predictive purposes) and because we were using a linear model. The data used for calculating degree-days was truncated at 65 degree-days for Pacific lampreys and at 52 degree-days for western brook lampreys because after this point the majority of eggs held at 22° C had hatched, resulting in an apparent change in survival that may be associated with developmental stage.

PRELIMINARY RESULTS AND DISCUSSION

Ammocoete identification

Validation of current diagnostic characteristics

Approximately half of the ammocoetes collected were identified to species, measured for length and mass, photographed, and sacrificed to provide genetic samples. Of the 50 individuals sampled in this manner, 42 were identified as Pacific lampreys and eight were identified as western brook lampreys based on current diagnostic characteristics (Appendix A). We are currently awaiting completion of genetic analyses, which will provide us with a measure of how accurate our preliminary identification was.

The remaining ammocoetes received individual marks and were sampled at intervals of approximately six weeks. Thus far, we have sampled individuals seven times. At each sampling event individuals were measured for length and mass, identified to species, metamorphosis stage was recorded, and photographs were taken (Figure 1). These data will allow us to track changes in individuals over time until completion of metamorphosis, and provide us with photographic records of these changes. We will also be able to examine inconsistencies in identification of individuals over time. To date there has been one case in which species identification has been inconsistent over time (Appendix B). In this case an individual was identified as Pacific lamprey the first four sampling events, western brook lamprey the fifth and sixth sampling event, and Pacific lamprey the seventh sampling event.

Morphometric description of laboratory spawned specimens

Individuals reared at different temperatures were collected over a period of 25 d. This time period encompassed a broad range of developmental stages from egg to

prolarva. Basic measurements have been taken for these individuals, including yolk and chorion diameter for eggs and notochord length for prolarvae (Appendix C). Samples have been preserved for future measurements and a database has been established for photographs of live specimens.

Effects of temperature on early life history stages

Temperature related mortality was rapid with the majority of loss occurring by the fifth day post fertilization for Pacific lampreys (Figure 2) and western brook lampreys (Figure 3). After the fifth day post fertilization, mortality at 22° C appeared to stagnate. This time period was loosely associated with the onset of hatching at 22° C and may indicate a significant change in the effect of temperature on survival based on developmental stage (Figure 2, Figure 3). Mean rates of mortality indicate the large difference between lampreys reared at 22° C and other treatments (Table 1, Table 2). However, by making comparisons among treatments based on number of days post fertilization we may be ignoring more ecologically relevant measures of time, such as the time required to reach particular developmental stages. Because temperature is likely to affect rates of development it may be more useful to use a metric based on developmental stage to examine the effects of temperature on survival.

Degree-day models take into account not only the time required to reach particular developmental stages, but also the temperature that organisms are exposed to during that time. Because of this, degree-days have been used extensively to predict the onset of particular developmental stages. Degree-days standardize time based on temperature making them a useful metric for this experiment. The effect of temperature on percent survival based on accumulated degree-days indicates that mortality rates are

greatly increased at 10 and 22° C when compared to other temperatures for Pacific lampreys (Table 3, Figure 4). Degree-day comparisons could not be made for western brook lampreys due to inconsistencies in sampling during early portions of this experiment. Inexperience in recognizing the difference between live/abnormal/dead eggs in the first three days of this experiment resulted in abnormal and dead eggs being categorized as abnormal. Therefore, it is not possible to separate abnormal from dead eggs prior to 52 degree-days. After 52 degree-days, criteria were established for positive identification of live and dead eggs and the dead eggs previously categorized as abnormal were removed. This resulted in what appears to be a drastic decline in survival between days three and four, but which is most likely a gradual decline in cumulative survival over the first four days of observation. Because the majority of western brook lampreys exposed to 22° C had already hatched, resulting in a drastic change in survival (see Figure 3), it was not possible to accurately estimate mortality rates in terms of degree-days.

Along with survival and mortality rates, developmental abnormalities were recorded daily. At this point it does not appear that temperature plays a significant role in the occurrence of abnormalities. Developmental abnormalities occurred at all temperatures and included deformed egg or yolk, fragmented yolk, bubble formation within the egg, and bent or deformed prolarvae (Figure 5). Developmental abnormalities observed were not always lethal, making them difficult to track through time. Because of this, there was a high degree of temporal variability in the number of abnormalities. Also, we did not have a systematic way of classifying abnormalities. This temporal

variability and the inability to classify abnormalities may be potential causes for the apparent lack of dependence on temperature.

FUTURE GOALS

We will continue our efforts of obtaining river lamprey specimens within the CRB. We were unable to locate them during 2000, and recorded observations are scarce and anecdotal. Our interest in including river lampreys in this study was to provide a diagnostic tool for lamprey identification within the CRB. Therefore, collecting river lamprey specimens from outside the CRB would have little utility in achieving our project goals.

Ammocoete identification

Validation of current diagnostic characteristics

We are currently awaiting completion of genetic analyses, which will give us an initial indication of how accurately we are able to identify ammocoetes of the CRB to the species level. This is critically important, as past work has indicated the inefficiency of current ammocoete diagnostic characteristics (USGS, unpublished data). We will also continue to sample ammocoetes currently held at the CRRL at intervals of approximately six weeks. This will allow us to follow known individuals through time and stages of metamorphosis. We will potentially be able to distinguish morphological changes and characteristics associated with various stages of metamorphosis for different species of lamprey, providing us with information to determine the validity of current diagnostic characteristics.

Morphometric description of laboratory spawned specimens

We will begin making morphometric descriptions of preserved samples that were collected in 2000 as well as collect more samples in 2001, resulting in an increased sample size and a better ability to distinguish morphometric differences between species.

Effects of temperature on early life history stages

We will repeat the experimental procedure used in 2000 with refinements in 2001. Our experience with this procedure should allow us to improve on sampling inaccuracies encountered during the early periods of this research. We will also develop a method to better categorize abnormalities in order to examine the effect of temperature not only on survival, but also on the occurrence of abnormalities. We will also be examining the validity of using different models to compare the effects of temperature on survival based on developmental stage, as this may be more ecologically relevant than survival based on absolute number of days. Time series data collected for morphometric descriptions of laboratory-spawned specimens should help us accomplish this goal by giving us an indication of the timing of different developmental stages.

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Table 1: Mean mortality rate over different time intervals (days post fertilization) of Pacific lampreys reared at 10, 14, 18, and 22° C.

Temperature	Mean mortality rate (percent mortality per day)		
	0-5 days	0-10 days	0-14 days
10° C	1.87	1.17	0.95
14° C	1.88	1.04	0.76
18° C	1.16	0.79	0.60
22° C	17.22	8.72	6.34

Table 2: Mean mortality rate over different time intervals (days post fertilization) of western brook lampreys reared at 10, 14, 18, and 22° C.

Temperature	Mean mortality rate (percent mortality per day)		
	0-5 days	0-10 days	0-14 days
10° C	7.33	4.44	3.37
14° C	2.63	2.44	1.81
18° C	4.30	2.74	2.04
22° C	19.00	9.88	7.05

Table 3: Mean mortality rate of Pacific lampreys reared at 10, 14, 18, and 22° C for 65 degree-days.

Mean mortality rate based on linear regression	
Temperature	(percent mortality per degree-day)
10° C	0.78
14° C	0.09
18° C	0.11
22° C	1.42

Figure 1: Sample photographs of ammocoete caudal region used for identification purposes based on Richards et al. (1982).

1a: Pacific lamprey distinguished by a slight lightening of pigment towards the caudal ridge (Richards et al. 1982).



1b: Western brook lamprey distinguished by a dark caudal region (Richards et al. 1982).

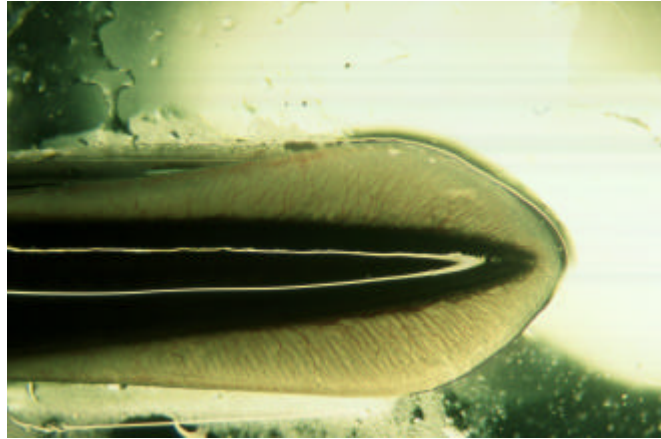


Figure 2: Effects of temperature on percent survival of Pacific lampreys reared at four temperatures (10, 14, 18, and 22° C) for 14 days post fertilization.

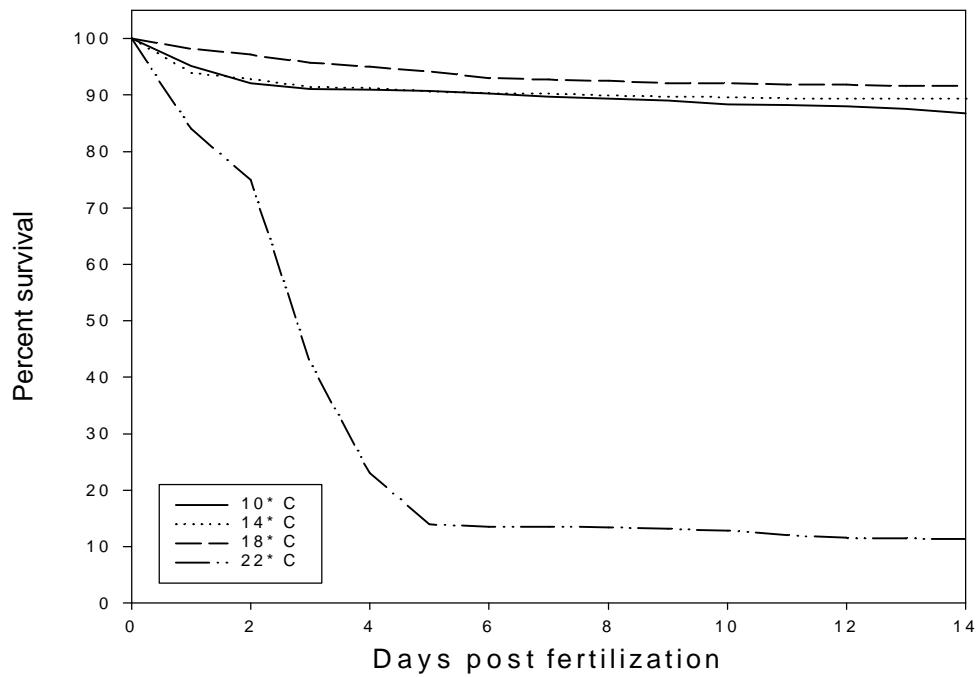


Figure 3: Effects of temperature on percent survival of western brook lampreys reared at four temperatures (10, 14, 18, and 22° C) for 14 days post fertilization.

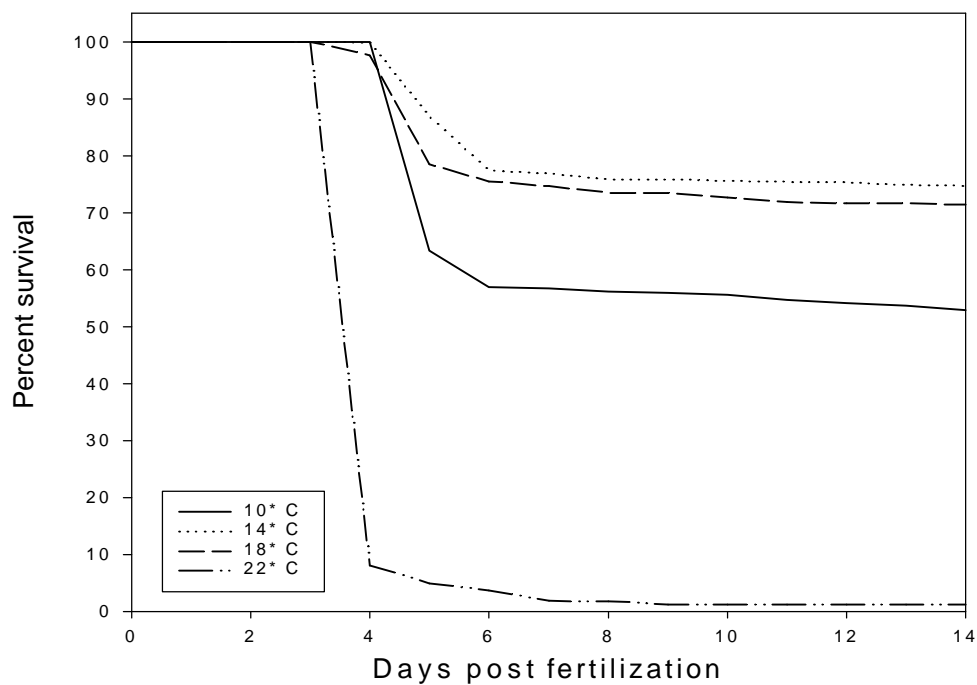


Figure 4: Effects of temperature on percent survival of Pacific lampreys reared at four temperatures (10, 14, 18, and 22° C) for 65 degree-days. Data points indicate mean percent survival and survival rates are based on linear regression.

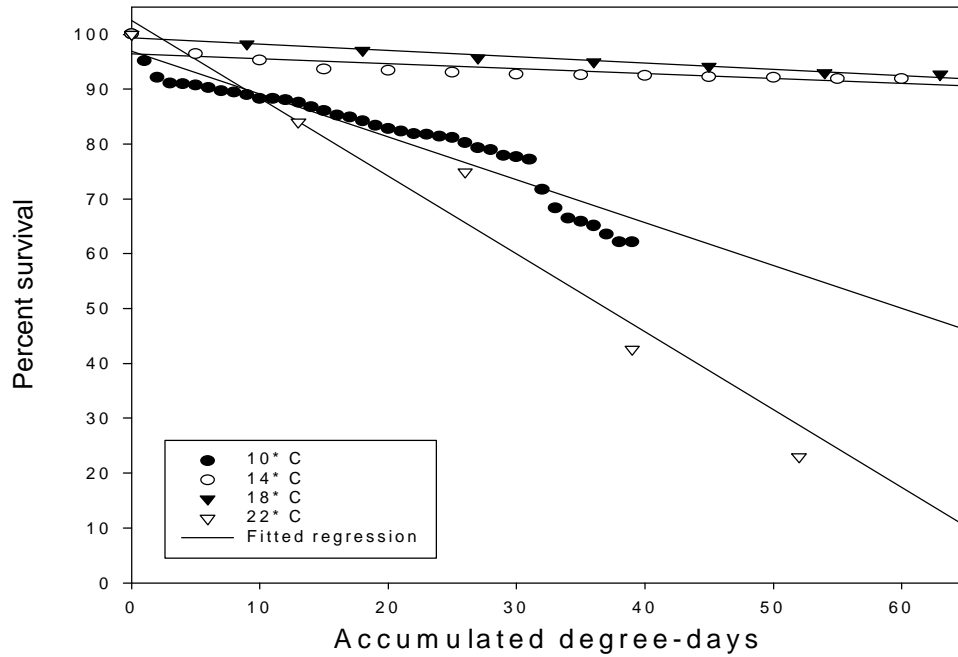
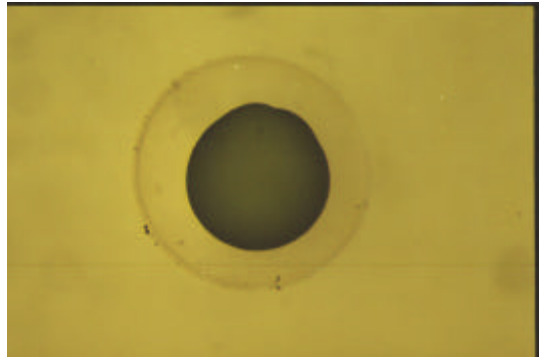
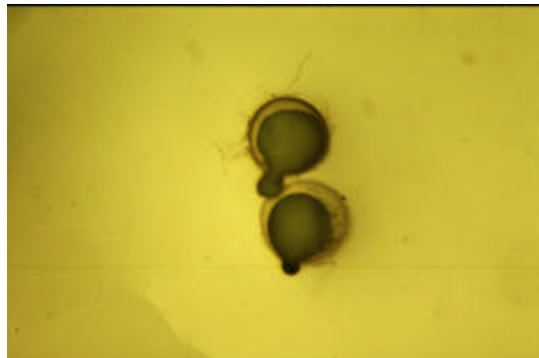


Figure 5: Photographic examples of: 1a) normal egg, 1b) two abnormal eggs, 1c) normal prolarvae, and 1d) abnormal prolarvae.

5a: Normal egg.



5b: Two eggs with abnormal (deformed) yolks.



5c: Normal prolarva.



5d: Abnormal prolarva with deformed or bent body.



Appendix A: Sample number, collection location, length, mass, and preliminary species identification for validation of current diagnostic characteristics for lamprey ammocoete identification. Genetic samples are currently being processed and confirmation of identification is not yet available (NYA). Collection location: ENT=Entiat River, JDW=John Day River and Walla Walla River, RED=Red River, CED=Cedar Creek. Preliminary species identification: PCL=Pacific lamprey, WBL=western brook lamprey.

Sample Number	Collection Location	Length (mm)	Mass (g)	Preliminary Species Identification	Genetic Confirmation
1	ENT	130	3.481	PCL	NYA
2	ENT	126	2.824	PCL	NYA
3	ENT	134	3.555	PCL	NYA
4	ENT	133	3.631	PCL	NYA
5	ENT	137	3.997	PCL	NYA
6	ENT	123	3.125	PCL	NYA
7	ENT	127	3.427	PCL	NYA
8	ENT	145	4.277	PCL	NYA
9	ENT	134	3.955	PCL	NYA
10	ENT	141	3.593	PCL	NYA
11	ENT	143	4.161	PCL	NYA
12	ENT	130	3.441	PCL	NYA
13	JDW	148	4.84	WBL	NYA
14	JDW	131	3.501	WBL	NYA
15	JDW	124	2.95	PCL	NYA
16	JDW	126	3.086	WBL	NYA
17	JDW	146	4.765	WBL	NYA
18	JDW	143	4.337	WBL	NYA
19	JDW	127	3.136	PCL	NYA
20	JDW	138	3.089	WBL	NYA
21	JDW	130	3.858	PCL	NYA
22	JDW	129	3.471	PCL	NYA
23	JDW	128	3.28	PCL	NYA
24	JDW	132	3.567	WBL	NYA
25	JDW	132	3.521	WBL	NYA
26	JDW	115	2.507	PCL	NYA
27	RED	141	4.56	PCL	NYA
28	RED	152	5.551	PCL	NYA
29	RED	141	4.543	PCL	NYA
30	RED	122	2.772	PCL	NYA
31	RED	111	2.19	PCL	NYA
32	RED	137	4.084	PCL	NYA
33	CED	117	2.28	PCL	NYA
34	CED	111	1.985	PCL	NYA
35	CED	104	1.587	PCL	NYA
36	CED	107	1.877	PCL	NYA
37	CED	108	1.749	PCL	NYA
38	CED	86	1.038	PCL	NYA
39	CED	119	2.474	PCL	NYA
40	CED	120	2.576	PCL	NYA
41	CED	119	2.439	PCL	NYA
42	CED	113	2.062	PCL	NYA
43	CED	97	1.201	PCL	NYA
44	CED	122	2.752	PCL	NYA
45	CED	116	2.595	PCL	NYA
46	CED	115	2.158	PCL	NYA
47	CED	107	1.768	PCL	NYA
48	CED	95	1.33	PCL	NYA
49	CED	96	1.316	PCL	NYA
50	CED	94	1.44	PCL	NYA

Appendix B: Thirty-one individuals have been repeatedly sampled over 10 months to examine the validity of current diagnostic characteristics. Minimum, maximum, and mean length (mm) are given for each individual. Preliminary identification at each sampling period is indicated: PCL=Pacific lamprey, WBL=western brook lamprey, NA=sample not taken due to mortality of specimen. Cases where preliminary identification was not consistent over time are indicated in **bold**.

Sample number	Length (mm)			Identification (based on current diagnostic characteristics)						
	Minimum	Maximum	Mean	24-Feb-00	04-Apr-00	26-May-00	28-Jun-00	03-Aug-00	05-Oct-00	01-Dec-00
51	74	101	89.57	PCL	PCL	PCL	PCL	PCL	PCL	PCL
52	104	124	113.71	PCL	PCL	PCL	PCL	PCL	PCL	PCL
53	88	96	91.80	PCL	PCL	PCL	PCL	NA	NA	NA
54	78	106	92.29	PCL	PCL	PCL	PCL	PCL	PCL	PCL
55	74	86	81.80	PCL	PCL	PCL	PCL	PCL	NA	NA
56	82	92	87.86	PCL	PCL	PCL	PCL	PCL	PCL	PCL
57	83	91	87.29	PCL	PCL	PCL	PCL	WBL	WBL	PCL
58	91	105	98.71	PCL	PCL	PCL	PCL	PCL	PCL	PCL
59	87	102	93.86	PCL	PCL	PCL	PCL	PCL	PCL	PCL
60	129	142	135.86	PCL	PCL	PCL	PCL	PCL	PCL	PCL
61	130	139	135.14	PCL	PCL	PCL	PCL	PCL	PCL	PCL
62	130	137	134.57	PCL	PCL	PCL	PCL	PCL	PCL	PCL
63	127	137	132.86	PCL	PCL	PCL	PCL	PCL	PCL	PCL
64	138	145	141.50	PCL	PCL	PCL	PCL	PCL	PCL	NA
65	140	150	144.14	PCL	PCL	PCL	PCL	PCL	PCL	PCL
66	127	132	130.71	PCL	PCL	PCL	PCL	PCL	PCL	PCL
67	126	133	129.75	PCL	PCL	PCL	PCL	PCL	PCL	PCL
68	108	121	114.29	PCL	PCL	PCL	PCL	PCL	PCL	PCL
69	124	130	127.57	PCL	PCL	PCL	PCL	PCL	PCL	PCL
70	132	138	134.86	PCL	PCL	PCL	PCL	PCL	PCL	PCL
71	128	143	138.29	PCL	PCL	PCL	PCL	PCL	PCL	PCL
72	122	125	123.57	PCL	PCL	PCL	PCL	PCL	PCL	PCL
73	129	132	130.71	PCL	PCL	PCL	PCL	PCL	PCL	PCL
74	125	133	129.43	PCL	PCL	PCL	PCL	PCL	PCL	PCL
75	121	128	125.14	WBL	WBL	WBL	WBL	WBL	WBL	WBL
76	118	124	121.57	WBL	WBL	WBL	WBL	WBL	WBL	WBL
77	120	126	123.71	WBL	WBL	WBL	WBL	WBL	WBL	WBL
78	122	131	127.14	PCL	PCL	PCL	PCL	PCL	PCL	PCL
79	125	129	127.29	PCL	PCL	PCL	PCL	PCL	PCL	PCL
80	116	130	123.00	PCL	PCL	PCL	PCL	PCL	PCL	PCL
81	115	123	119.29	WBL	WBL	WBL	WBL	WBL	WBL	WBL

Appendix C: Collection date, temperature reared at, species, number of live individuals, number of dead individuals, number of abnormal individuals, mean yolk diameter, mean chorion diameter, and mean notochord length of Pacific lamprey and western brook lamprey collected for morphometric description. Samples lacking either mean yolk diameter and mean chorion diameter or mean notochord length indicate that individuals sampled that day did not contain either eggs or prolarvae respectively. (Species: PCL=Pacific lamprey, WBL=western brook lamprey)

Collection date	Temperature (° C)	Species	Number live	Number dead	Number abnormal	Mean yolk diameter (mm)	Mean chorion diameter (mm)	Mean notochord length (mm)
6/16/00	10	PCL	18	2	3	1.98	2.31	
6/16/00	14	PCL	20	0	9	2.19	2.57	
6/16/00	18	PCL	18	2	3	1.98	2.58	
6/16/00	22	PCL	4	16	0	2.16	2.64	
6/20/00	10	PCL	18	2	5	1.78	2.31	
6/20/00	14	PCL	17	3	0	1.90	2.37	
6/20/00	18	PCL	17	3	1	1.28	1.66	
6/20/00	22	PCL	0	20	20	1.34	1.72	
6/24/00	10	PCL	16	4	4	1.86	2.27	
6/24/00	14	PCL	14	6	0	2.16	2.49	
6/24/00	18	PCL	20	0	0			5.15
6/24/00	22	PCL	4	16	1			5.17
6/28/00	10	PCL	18	2	0	1.34	1.75	
6/28/00	14	PCL	20	0				5.33
6/28/00	22	PCL	0	20	20	1.24	1.74	
6/30/00	10	WBL	20	0	1			5.56
7/3/00	10	PCL	18	2	2	1.34	1.60	
7/3/00	14	PCL	20	0	0			6.92
7/6/00	10	PCL	19	1	2	1.37	1.54	
7/6/00	14	PCL	20	0	0			7.12
7/10/00	10	PCL	13	7	0			4.27